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Risk Assessment Branch VI, Health Effects Division (7509P) **Date:** 04/23/2019

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DATA EVALUATION RECORD

STUDY TYPE: Special Study – Assessment of Acoustic Startle Response in Juvenile and Adult Rats Following a Single Oral Dose of Deltamethrin.

PC CODE: 097805 **DP BARCODE:** D444188

TEST MATERIAL (PURITY): Deltamethrin technical (99.9%)

SYNONYMS: DLM

CITATION: Vorhees, C., and Williams., M. (2016) Acute Neurotoxicity of Deltamethrin in Juvenile (PND15) and Adult Rats Assessed by Acoustic Startle and Detailed Neurological Clinical Observations. Cincinnati Children's Research Foundation Div. of Neurology Neuroscience Laboratory. Laboratory ID: Deltamethrin Rat Juvenile-Adult Neurotoxicity Study. April 13, 2016. MRID 50409301. Unpublished.

SPONSOR: Council for the Advancement of Pyrethroid Human Risk Assessment, LLC (CAPHRA) C/o Consumer Specialty Products Association

EXECUTIVE SUMMARY:

Several preliminary studies (summarized below) were performed to establish the suitability of acoustic startle response (ASR) to measure acute neurotoxicity effects in adult and juvenile rats and to establish doses for the definitive studies. The purpose of the definitive studies was to determine dose response. In the definitive studies, deltamethrin was administered to both adult (0, 2, 8, or 25 mg/kg deltamethrin [nominal doses]) and PND 15 rats (0, 1, 2, or 4 mg/kg deltamethrin [nominal doses]) in 5 mL/kg of corn oil. Measured (internal) doses were not provided for the definitive study. Both adults (16/males per group) and juveniles (16/sex/group, 1/sex/litter) were then subjected to detailed clinical observations and acoustic startle responses were measured at 2, 4, 6 and 8 hrs after administration.

No mortality was observed in the definitive experiments. Clinical observations were limited in adult rats. None of the clinical observations rose above moderate in the 2 and 8 mg/kg deltamethrin groups, and only salivation (1/16) and motility (5/16) rose above moderate in the 25 mg/kg deltamethrin group. The peak effect for salivation and motility after exposure to 25 mg/kg deltamethrin occurred at 2.5 h and 4 h, respectively. After reanalysis of the data by HED statisticians, only the high dose group (25 mg/kg) was significantly different from the control

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and only at 2 hours and 4 hours following the treatment.
There were more detailed clinical observations in PND15 rats compared to adult rats even at lower doses, indicating that young rats are more susceptible to deltamethrin neurotoxicity than adults. None of the clinical observations rose above moderate in the 1 and 2 mg/kg deltamethrin groups, but rose above moderate for motility, salivation (6/17 for females and 6/17 for males) and tremor (2/17 for females) in the 4 mg/kg deltamethrin group. However, these increases in the 4 mg/kg deltamethrin group did not correspond to the decreases in ASR. In PND15 pups, deltamethrin induced significant dose-dependent decreases in ASR that did not dissipate or level-off by 8 h post-treatment. Therefore, it is not possible to determine the LOAEL or NOAEL for the PND15 pups, since it is less than 1.0 mg/kg deltamethrin. In order to determine the threshold, doses of 0.125, 0.25, and 0.5 mg/kg deltamethrin should be assessed.

The study indicates that treatment with deltamethrin decreased the acoustic startle response in PND 15 and adult rats. It also suggests that PND15 pups are more sensitive to the neurotoxic effects of deltamethrin based on clinical observations. However, the available data did not demonstrate an appropriate magnitude or time-course of effects on acoustic startle in PND 15 rats. Appropriate doses for PND 15 pups were not established, which in turn did not allow study authors to establish a clear time to peak effect, or post-treatment interval for testing. Therefore, a quantitative comparison between juveniles and adults is not appropriate using ASR data in this study.

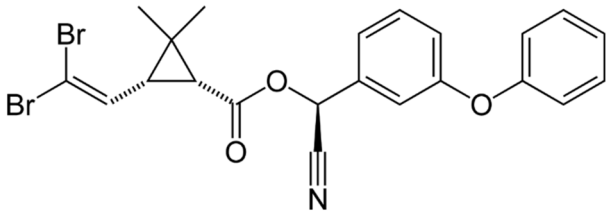
Based on the effects observed in this study, the adult LOAEL was 25 mg/kg bw based on decreased ASR, with a NOAEL of 8 mg/kg bw/day. The juvenile LOAEL was 1 mg/kg bw based on decreased ASR, with a NOAEL that could not be established.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Deltamethrin Technical

Description: technical, white solid
Lot/batch #: ABKBDCK008
Purity: 99.9% a.i.
CAS # of TGAI: 52918-63-5
Structure:



2. Vehicle and/or positive control: Reagent-grade corn oil (dosing volume of 5 mL/kg, unless otherwise specified)

3. Test animals:

Species:	Rat	
Strain:	Sprague-Dawley (SD)	
Age/weight at dosing:	PND 15 in juvenile studies 12-13 weeks in adult studies	
Source:	Charles River - Raleigh, NC	
Housing:	2 rats/cage	
Diet:	Certified Rodent NIH 007 diet *ad libitum*	
Water:	Deionized and reverse osmosis-filtered, *ad libitum*	
Environmental conditions:	**Temperature:**	19±2°C
	Humidity:	50%±20%
	Air changes:	20/hr
	Photoperiod:	10 hrs dark/ 14 hrs light
Acclimation period:	N/A	

B. PRELIMINARY STUDIES:

1. Adult Preliminary Studies:

ASR Dose-Range Finding Experiments:

Study 1:

Objective: The objective of this experiment was to determine a possible starting dose of deltamethrin.

Methods: In Experiment 1, 4 groups of 12 male Sprague-Dawley rats (9 weeks old) were exposed to 0, 2, 4, or 8 mg/kg of deltamethrin by gavage. The control group received a dose of vehicle (3 mL/kg corn oil). ASR measurements were conducted at 2 h post-treatment (100 trials per test session). Detailed clinical observations were not collected.

Results/Investigator's Conclusions: ASR was measured in adult rats at doses of 1.2, 2.4, or

4.8 mg/kg deltamethrin in 3 mL/kg corn oil (nominal doses: 2, 4, or 8 mg/kg deltamethrin). Decreased ASR was observed for all time points and doses (Figure 4). The largest difference in ASR between the control and treatment groups occurred at 2 h post-treatment (~48% decrease). The decrease in ASR due to deltamethrin exposure appears to be short-lived, possibly only lasting 2 h post-treatment. ASR after exposure to 4.8 mg/kg was significantly different than the controls at the 2 h post-treatment observation only. No other dose or time was statistically significant. Several changes were then made in subsequent experiments to detect reliable deltamethrin-induced ASR reductions in adult male rats including. The use of 100 trials/session may be causing more habituation than was desirable, so 50 trials/session was used in later experiments to reduce time-dependent habitation.

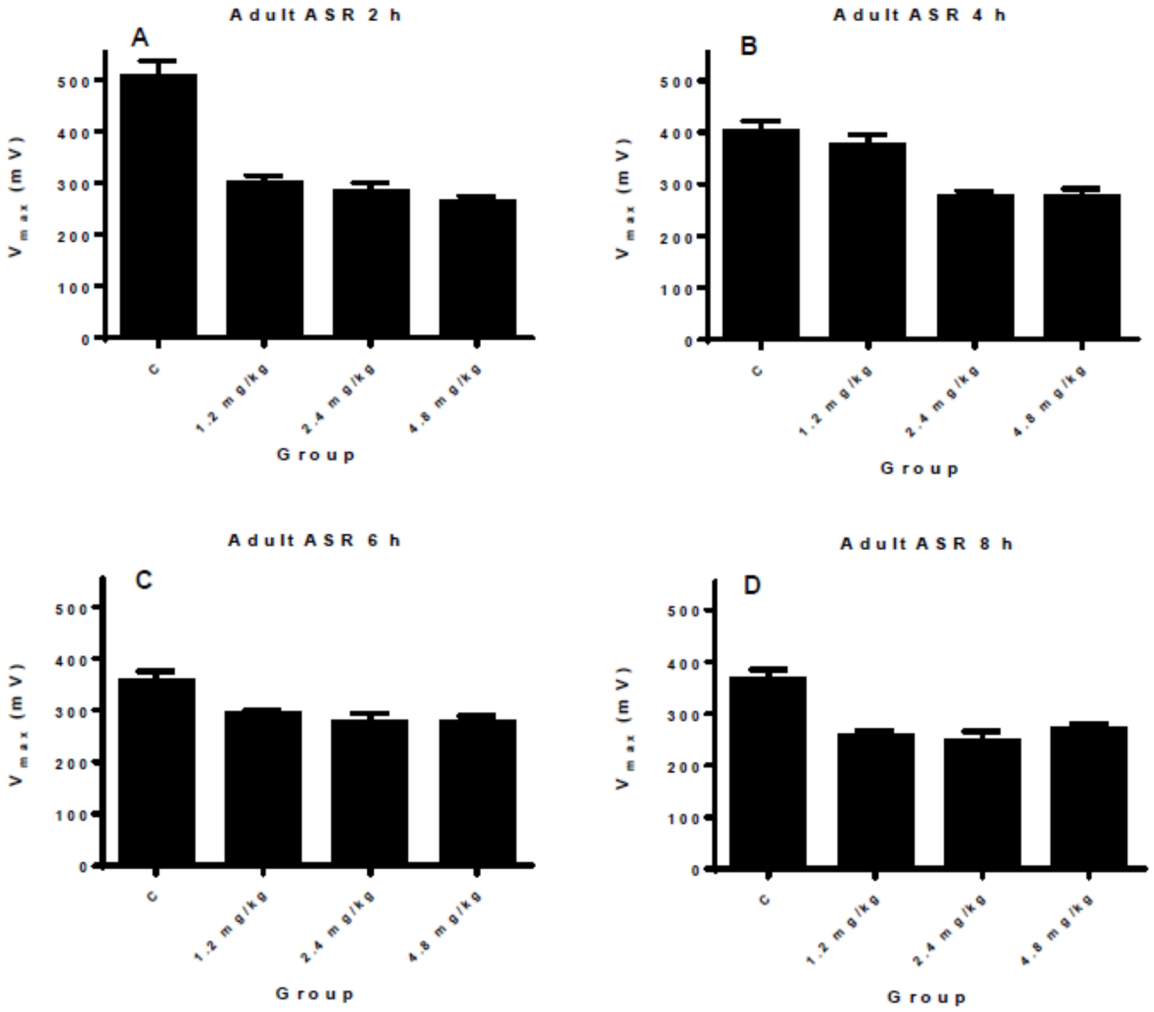


Figure 1 Adult ASR 2, 4, 6, and 8 h post-treatment after exposure to deltamethrin by gavage in corn oil. (MRID 50409301; Figure 7; page 30).

Study 2:

Objective: The objective of this experiment was to evaluate if published results using a dose volume of 1mL/kg corn oil could be replicated.

Methods: In Experiment 2, 4 groups of 12 male Sprague-Dawley rats (9 weeks old) were

exposed to 8 mg/kg of deltamethrin by gavage with a dose volume of 1 mL/kg corn oil. ASR measurements were conducted at 2, 4, and 6 h post-treatment (100 trials per test session). Detailed clinical observations were not collected.

Results/Investigator's Conclusions: In Experiment 2, 8 mg/kg deltamethrin in 1 mL/kg corn oil decreased ASR in the adult male SD rat, with a peak reduction of V_{max} (71.6%) at 4 h post-treatment.

Study 3:

Objective: The objective of this experiment was to evaluate if a response using 5 mL/kg corn oil could be measured.

Methods: In Experiment 3, 4 groups of 12 male Sprague-Dawley rats (9 weeks old) were exposed to 8 mg/kg of deltamethrin by gavage with a dose volume of 5 mL/kg corn oil. ASR measurements were conducted at 2, 4, 6, and 8 h post-treatment (100 trials per test session). Detailed clinical observations were not collected.

Results/Investigator's Conclusions: In Experiment 3, 8 mg/kg deltamethrin in 5 mL/kg corn oil decreased ASR in the adult male SD rat; however, there was not a time-dependent decrease as that observed in 1 mL/kg corn oil.

2. Juvenile Rats

Tolerability Dose-Range Finding Experiments

Methods: PND15 rats were exposed by gavage to the following nominal doses: 1) 6 or 8 mg/kg deltamethrin (measured doses of 3.6 or 4.8 mg/kg) (Experiment 1); 2) 3, 4, or 5 mg/kg deltamethrin (measured doses of 1.8, 2.4, or 3 mg/kg) (Experiment 2); and 3) 1, 2, or 4 mg/kg deltamethrin (measured doses of 0.6, 1.2, or 2.4 mg/kg) (Experiment 3)[1]. Detailed clinical observations were conducted at 1, 2, 3, 4, 5, 6, 7, and 24 h post-treatment; in subsequent experiments an 8 h observation time replaced the 24 h observation. Detailed clinical observations were performed on all animals. The CHECKED (X) parameters were examined.

[1] The data from the juvenile preliminary experiments were used to determine definitive doses, but a technician made an error in administering the compound in several of the preliminary experiments. She measured out the proper amount of compound for each dose level and mixed it in the proper volume of corn oil, but when she gavaged animals she used a dosing schedule that she had transferred from another study that was based on 3 ml/kg rather than 5 mL/kg. Hence, she administered 60% of the intended dose.

	HANDLING OBSERVATIONS
X	Lacrimation* / chromodacryorrhea
X	Salivation*
X	Tremors when held
X	Muscle tone*
X	Ventral wetness
	SENSORY OBSERVATIONS
X	Tail pinch response
	PHYSIOLOGICAL OBSERVATIONS
	Body weight*
X	Body temperature+ (subjectively determined, not by thermometer)
X	**OPEN FIELD OBSERVATIONS**
X	Mobility/Motility
X	Convulsions*
X	Tremors*
X	Gait abnormalities / posture*
X	Gait score*
X	Compulsive Licking and/or biting
X	Writhing
X	Other

***ASR Dose-Range Finding Experiments*:**

Methods: PND15 male rats were exposed to 1) 0, 3, 4, or 5 mg/kg deltamethrin (measured doses of 0, 1.8, 2.4, or 3 mg/kg) (Experiment 1) or 2) 0, 1, 2, or 4 mg/kg deltamethrin (measured doses of 0, 0.6, 1.2, or 2.4 mg/kg) (Experiment 2). The control group received a dose of 3 mL/kg corn oil. Twelve litters with 10 males per litter; 2 pups per dose per litter were examined. Litters were tested on the day they turned PND15.

Results/Investigator's Conclusions: Mortality was not affected at the lowest two doses (1.2 and 1.8 mg/kg) but increased in a dose-dependent manner. Additionally, there was an increase in mortality at doses higher than 3 mg/kg. In all cases, mortality was delayed as it began to emerge 4 h post-treatment in the two highest dose groups and at 5-6 h post-treatment at the lower doses.

TABLE 1. Mortality in PND15 male rats.

Time (h)	Dose Level (mg/kg bw)					
	1.2	**1.8**	**2.4**	**3**	**3.6**	**4.8**
1	0/15	0/15	0/15	0/15	0/30	0/30
2	0/15	0/15	0/15	0/15	0/30	0/30
3	0/15	0/15	0/15	0/15	1/30 (3.3%)	0/30
4	0/15	0/15	0/15	0/15	4/30 (13.3%)	9/30 (30%)

TABLE 1. Mortality in PND15 male rats.						
Time (h)	Dose Level (mg/kg bw)					
	1.2	1.8	2.4	3	3.6	4.8
5	0/15	0/15	1/15 (6.7%)	2/15 (15.4%)	6/30 (20%)	14/30 (46.7%)
6	0/15	1/15 (6.7%)	1/15 (6.7%)	2/15 (15.4%)	13/30 (43.3%)	23/30 (76.7%)
7	0/15	2/15 (15.4%)	1/15 (6.7%)	2/15 (15.4%)	15/30 (50%)	29/30 (96.7%)
8	0/15	2/15 (15.4%)	1/15 (6.7%)	2/15 (15.4%)	22/30 (73.3%)	30/30 (100%)

Data reorganized from MRID 50409301; Table 8; page 26.

Dose-range finding studies were conducted to determine if effects to ASR could be detected in the absence of adverse neurological symptoms. Based on the results, it is not possible to separate the two phenomena at the doses tested but the effects on the detailed clinical observations could be minimized at the doses that affected ASR. In the first study, juvenile (PND15) rats were exposed to doses of 0, 1.8, 2.4, or 3 mg/kg deltamethrin in 3 mL/kg corn oil. Mortality occurred in the 3 mg/kg deltamethrin exposure, so a second study was conducted with lower doses of 0, 0.6, 1.2, and 2.4 mg/kg deltamethrin in 3 mL/kg corn oil. The data between the 2 studies could be pooled since the 2 control groups and the two 2.4 mg/kg groups did not differ significantly from one another at any of the 4 test intervals. The data for the Control and 2.4 mg/kg/ groups from the 2 experiments were combined and new statistics were conducted. Significantly decreased ASR was observed at 2 h post-treatment in the 1.8, 2.4, and 3 mg/kg deltamethrin groups. At 4, 6, and 8 h post-treatment, there is a dose-dependent effect to ASR (Figure 2). At 8 h post-treatment, the 0.6 mg/kg deltamethrin groups showed a trend ($p<0.06$) suggesting that with larger group sizes, significant effects may be detected at this dose in the absence of detailed clinical observation effects.

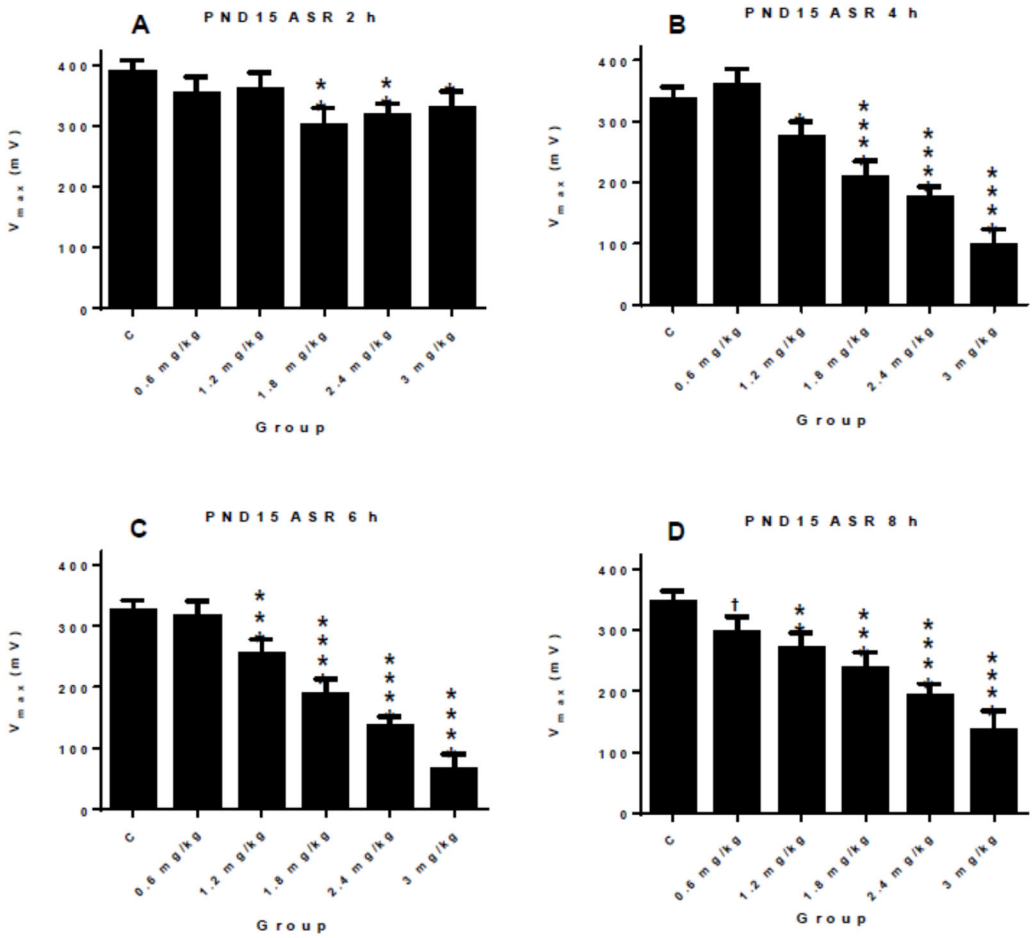


Figure 2. PND 15 ASR 2, 4, 6, and 8 h post-treatment after exposure to deltamethrin by gavage in corn oil. (MRID 50409301; Figure 10; page 37).

Adults and Juveniles (PND15)

***Detailed Clinical Observation Experiments (Adults)*:**

Study 1

Objective: The objective of this experiment was to determine how to examine the detailed clinical observations.

Methods: In Experiment 1, adult male rats were exposed to 2, 4, or 8 mg/kg deltamethrin by gavage. No symptoms were observed, so the dose was increased to nominal concentrations of 25, 35, or 45 mg/kg deltamethrin by gavage (actual concentrations: 15, 21, 27 mg/kg deltamethrin in 3 mL/kg corn oil).

Study 2

Objective: The objective of this experiment was to determine how to examine the detailed clinical observations.

Methods: In Experiment 2, adult male rats were exposed to nominal concentrations of 55, 65, or 75 mg/kg deltamethrin by gavage (actual concentrations: 33, 39, or 45 mg/kg deltamethrin in 3 mL/kg corn oil). These doses were used to set a revised high nominal dose of 25 mg/kg (actual concentration: 15 mg/kg in 3 mL/kg corn oil) for the definitive experiment, since this dose induced a mild level of symptoms.

Detailed clinical observations were performed on all juvenile animals in the tolerability experiments described above.

Results/Investigator's Conclusions: Detailed Clinical Observations were conducted on both juveniles (PND15) and adults. Salivation had an earlier onset and longer duration in adults than in PND15 rats, but the dose in adults was >20X higher (Figure 3). Salivation was not observed in juvenile rats exposed to 1.2 mg/kg but a "mild" level of salivation peaked at 3 h in juvenile rats exposed to 2.4 mg/kg. In adult rats, exposure to 15 and 21 mg/kg deltamethrin induced clear increases in salivation, which reached moderate at the peak of 4 h post-treatment after an exposure to 21 mg/kg deltamethrin. However, these doses are ≥10x higher that doses used in the juvenile study. In both juvenile and adult rats, all signs of salivation ended by 8 h post-treatment.

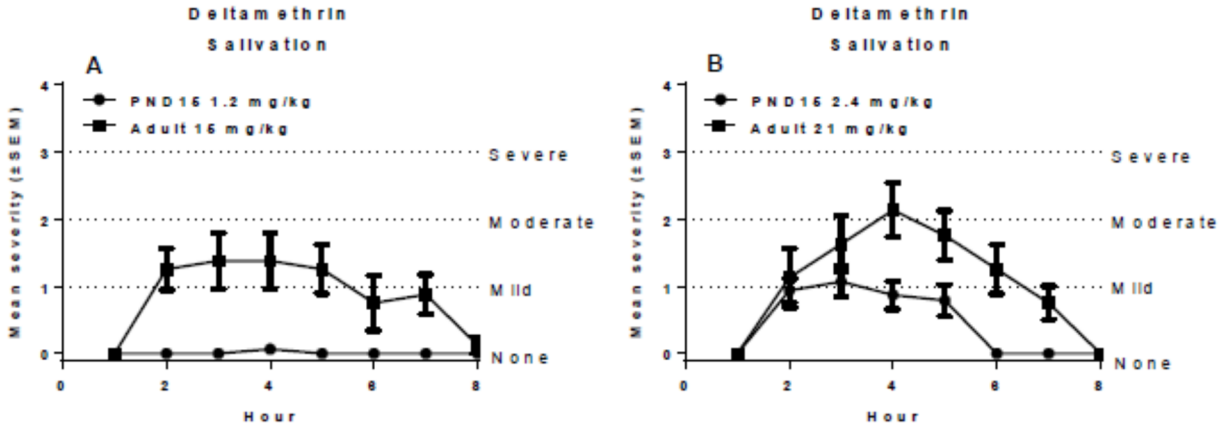


Figure 3. Salivation in juvenile (PND15) and adult rats. (MRID 50409301; Figure 4; page 28).

Motility was affected in a similar pattern in juveniles and adults exposed to 1.2 mg/kg and 15 mg/kg, respectively (Figure 4A). Impaired motility increased by 2 h post-treatment and commenced by 8 h post-treatment for adults exposed to 15 mg/kg deltamethrin but continued throughout the post-treatment observations for juveniles exposed to 1.2 and 2.4 mg/kg as well as adults exposed to 21 mg/kg deltamethrin (Figure 4). At the peak, impaired motility was slightly higher than mild but never reached moderate. In adults, impaired motility reached moderate at the peak of 4 h post-treatment after exposure to 21 mg/kg deltamethrin.

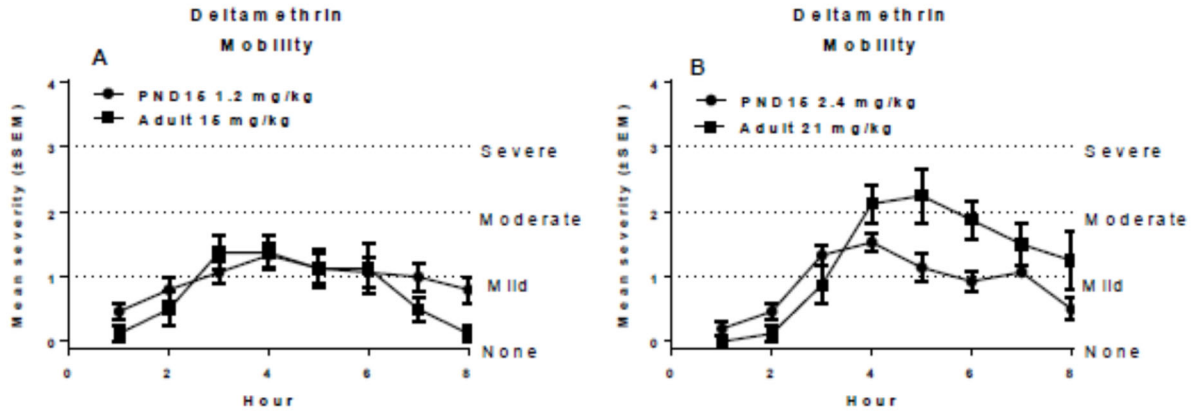


Figure 4. Motility (described as mobility in figure) in juvenile (PND15) and adult rats (MRID 50409301; Figure 5; page 28).

Visual tremors as well as tremors when held were assessed. Visual tremors proved more reliable than held tremor and is presented in the report. Mild visual tremors were observed in both juvenile (PND15) and adult rats (Figure 5). Tremors peaked at 3-4 h post-treatment in adult rats exposed to 15 mg/kg deltamethrin. In juvenile (PND15) rats, tremor ratings were slightly higher and lasted longer, with a few remaining at 8 h post-treatment compared to adult rats.

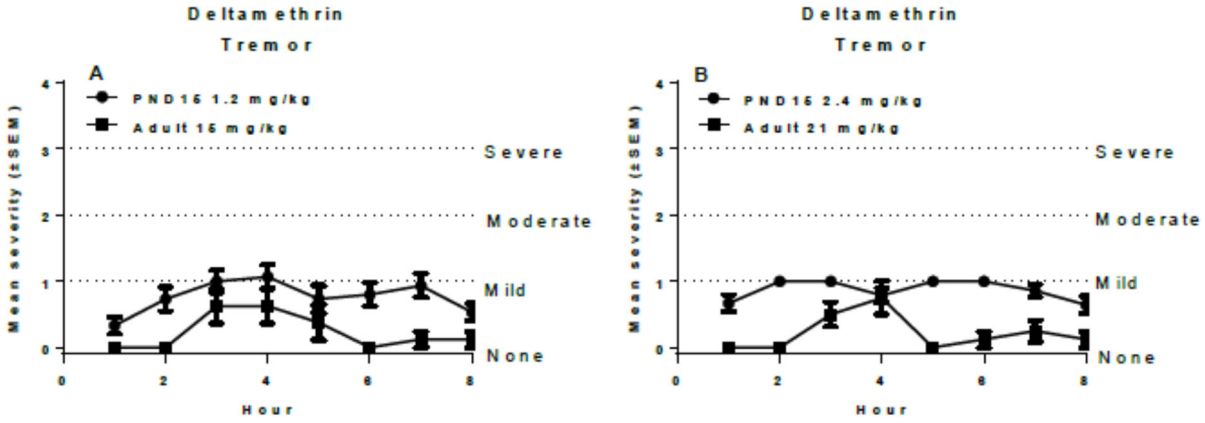


Figure 5. Visual tremors in juvenile (PND15) and adult rats. (MRID 50409301; Figure 6; page 29).

Plasma and Whole-brain tissue concentration

Objective: The objective of this experiment was to determine plasma and whole-brain tissue deltamethrin concentrations in adults and juvenile (PND15) rats.

Methods: Separate groups of rats were treated and used to provide plasma and whole-brain tissue samples for analysis. PND15 male rats were exposed to 1, 2, or 4 mg/kg deltamethrin by gavage. Adult male rats were exposed to 0, 2, 8, or 25 mg/kg deltamethrin by gavage. All rats were sacrifice by decapitation at 2, 4, 6, or 8 h post-dosing. Tissues were collected from 3 litters (18 pups stratified by dose) for each time point. Following sacrifice by decapitation,

blood was collected in heparinized tubes. A 0.64 M concentration of sodium fluoride (~100 µl/ml of blood) was added to each sample to inhibit carboxylesterases that catalyze hydrolysis of pyrethroids. The blood was centrifuged at 4500 rpm for 15 min to separate the RBC and other components. The plasma was transferred to plastic tubes, frozen, and stored at -80ºC until shipped to the analytical laboratory. The whole brain was removed and snap-frozen in isopentane, placed in tubes, and stored at -80ºC until shipped to the analytical laboratory. These data were used to determine the definitive doses; however, only 60% of the intended doses was administered do to a technical error.

Results/Investigator's Conclusions: At equivalent doses, plasma and brain concentrations in PND15 rats were higher than in adult rats (Table 2). Increased plasma levels as well as increased uptake of deltamethrin in juvenile rats was observed. The differences between dose groups at both ages were statistically significant. Brain-to-plasma ratios at both ages show that brain concentrations increase as plasma deltamethrin concentrations increase. Additionally, brain-to-plasma ratios are greater in PND15 rats than in adult rats, indicating that PND15 rats take up deltamethrin into the brain to a greater extent than adult rats. At lower plasma concentrations, the relative amount of brain uptake is greater than at higher plasma concentrations. Brain concentrations and ASR changes do not parallel one another over time (Figure 6). Plasma and brain deltamethrin concentrations peak at 4 h post-treatment while ASR peaks at 6 h post-treatment in PND15 rats.

Table 2. Brain and plasma concentrations of deltamethrin in juvenile (PND15) and adult rats

	PND15				Adult		
Dose (mg/kg)	0.6	1.2	2.4		1.2	2.4	4.8
Brain							
Time (h)							
2	11.3±1.5 (6)	25.4±1.6 (7)	38.0±2.4 (5)		5.3±0.8 (6)	8.6±2.3 (6)	13.0±2.8 (7)
4	27.2±3.5 (6)	48.2±5.3 (6)	109.7±9.0 (6)		8.1±1.8 (6)	14.7±2.6 (6)	18.8±1.4 (5)
6	14.8±0.8 (6)	30.0±3.9 (6)	92.2±12.8 (4)		6.1±1.1 (6)	11.6±2.2 (6)	16.8±2.2 (6)
8	9.6±0.8 (6)	19.0±2.3 (6)	62.1±11.1 (6)		5.3±0.3 (6)	8.5±1.4 (6)	13.8±2.2 (6)
Plasma							
2	340.3±33.4 (6)	687.9±65.3 (7)	1258.6±187.1 (5)		355.8±92.5 (6)	541.0±105.3 (6)	556.5±154.7 (5)
4	252.3±11.3 (6)	460.7±36.4 (6)	1551.5±119.6 (6)		242.9±35.3 (6)	535.8±115.5 (6)	525.5±142.5 (5)
6	78.2±6.2 (6)	243.8±52.6 (6)	1330.8±250.7 (4)		117.0±19.3 (6)	348.5±67.6 (6)	499.5±89.5 (6)
8	44.3±8.7 (6)	102.2±10.8 (6)	610.7±187.2 (6)		57.4±9.9 (6)	225.1±81.5 (6)	276.0±34.3 (6)

(From MRID 50409301; Table 13; page 39)

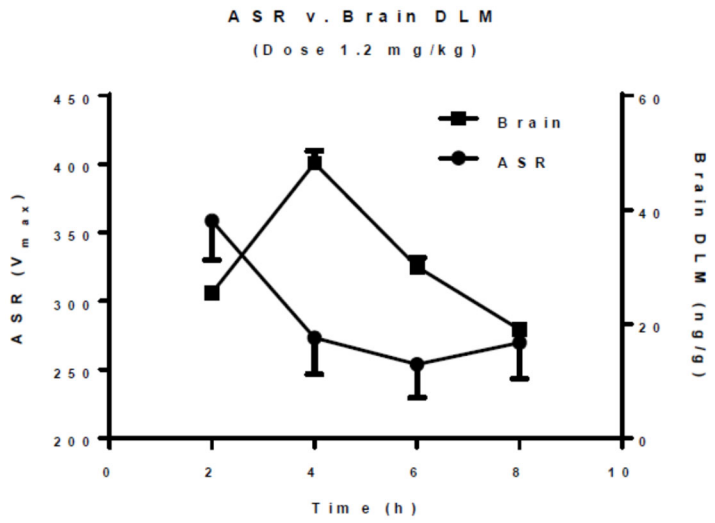


Figure 6. Comparison of ASR to brain deltamethrin concentration in separate rats at PND15. (MRID 50409301; Figure 13; page 41).

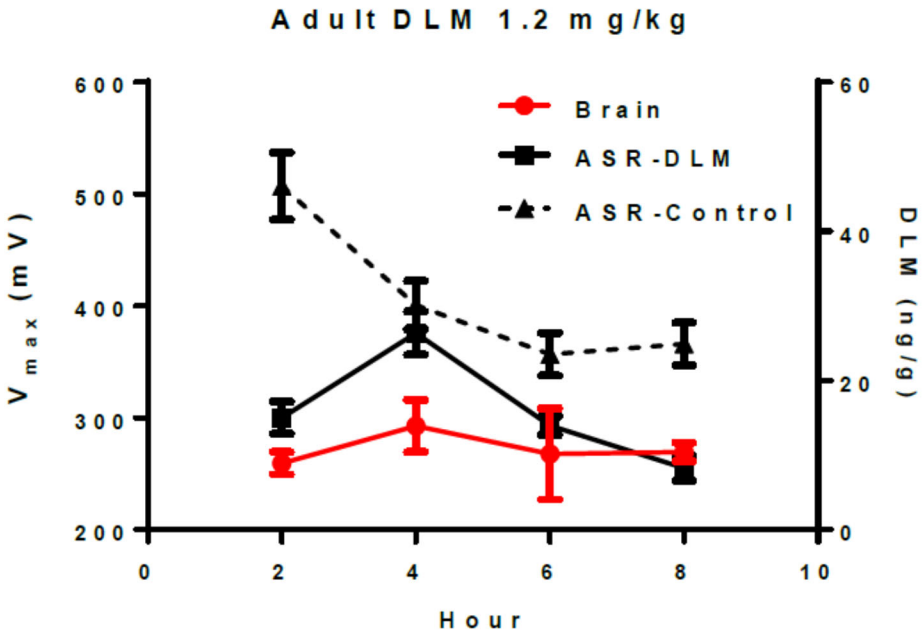


Figure 7. Comparison of adult (left axis) and brain (right axis) from treatment to 1.2 mg/kg deltamethrin. (MRID 50409301; Figure 14; page 42).

Tactile Startle Response (TSR):

Objective: The objective of this experiment was to determine if a larger response could be obtained for the startle response by using a tactile stimulus rather than an acoustic one.

Methods (Adults): In this experiment, 24 adult male Sprague-Dawley rats were divided into two groups, treated by gavage (3 mL/kg) with 0 or 4.8 mg/kg deltamethrin and tested 2, 4, 6, or 8 h later for startle for 100 trials and analyzed in 10-trial blocks. The ITI was 20 s as in previous experiments, but the trial types were mixed (10-trial blocks each of acoustic and

tactile stimuli were alternated). The acoustic trials used a 120 dB SPL mixed frequency stimulus, as in other experiments, and the tactile trials used an air-puff (80 psi) alternating ASR and TSR in 10-trial blocks (100 trials).

Methods (Juvenile): For this experiment, Sprague-Dawley pups were treated by gavage (3 mL/kg) with 0 or 1.2 mg/kg deltamethrin and tested 2, 4, 6, or 8 h later for startle for 100 trials and analyzed in 10-trial blocks. The ITI was 20 s as in previous experiments, but the trial types were mixed (10-trial blocks each of acoustic and tactile stimuli were alternated). The acoustic trials used a 120 dB SPL mixed frequency stimulus, as in other experiments, and the tactile trials used an air-puff (80 psi) alternating ASR and TSR in 10-trial blocks (100 trials). These trials were conducted in litters without control for litter effects in order to determine how PND15 pups would respond to the air-puff stimulus.

Results/Investigator's Conclusions: TSR has the potential to detect deltamethrin-induced reductions as or more clearly than ASR. Deltamethrin (1.2 mg/kg) significantly decreased TSR for 8 h (Figure 8). The effects of deltamethrin to ASR are short-lived, which is a similar pattern to the results of the adult TSR (Figure 9). If TSR were to be used, further testing will be required to ensure its sensitivity across a range of doses.

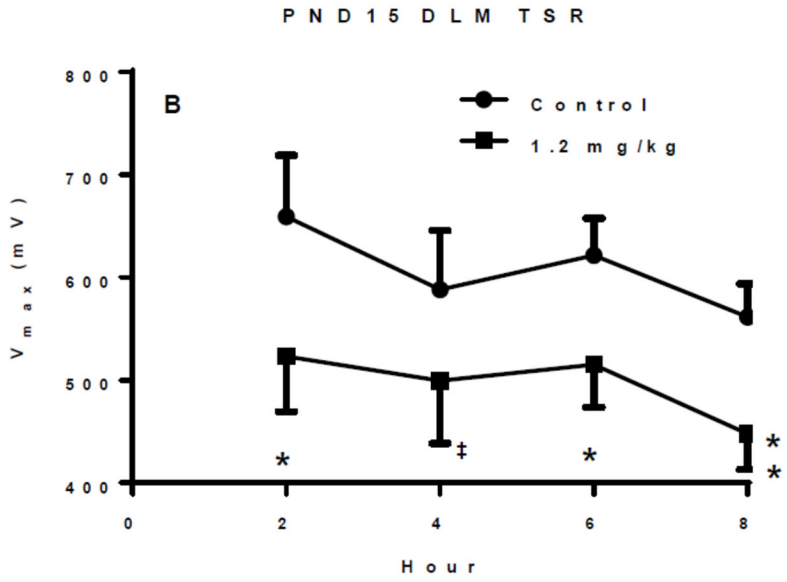


Figure 8. Effects of 1.2 mg/kg deltamethrin on TSR in PND15 pups. (MRID 50409301; Figure 16; page 43).

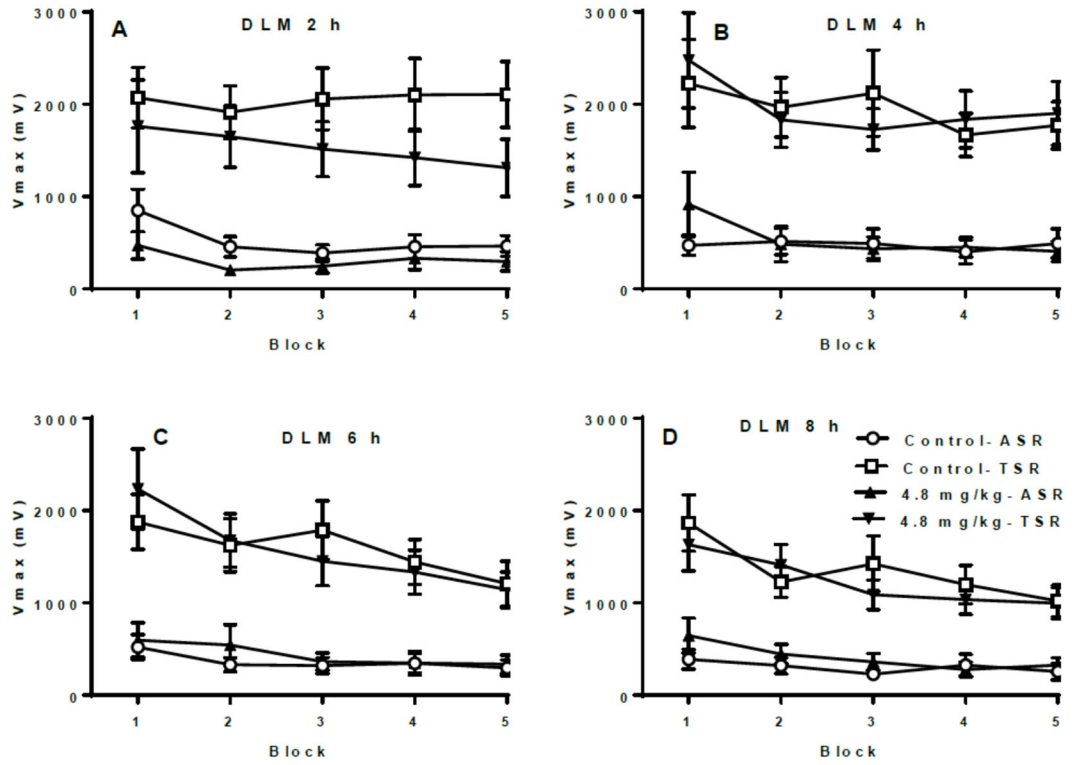


Figure 9. Effects of deltamethrin in adult rats given ASR and TSR in the same session. (MRID 50409301; Figure 15; page 42).

EPA Reviewer Comments on Adult and Juvenile Preliminary Data: The reviewer agrees with the general conclusions of all preliminary studies. The reviewer agrees that ASR decreased in both adults and PND15 pups; however, it is unclear to the reviewer why 0.6 mg/kg deltamethrin was not assessed in the definitive study, since this is a clear NOAEL in the preliminary study.

D. DEFINITIVE STUDY DESIGN:

1. In life dates: N/A

2. Animal assignment and treatment:

One to three days prior to treatment, adult rats were weighed prior to group assignment. The adult rats were assigned to groups balanced for ASR using a pretest. Juvenile groups were also ASR pretested on PND14 but matching within litters was not feasible. For adult rats, ASR Means and SDs were used to match groups for ASR V_{max} values. Juvenile rats were assigned to groups in the same manner on PND14 with no variation in day allowed. The use of ASR pretesting was based on preliminary data showing that body weight was not significantly correlated to ASR whereas ASR is correlated with itself across days with repeated testing. Therefore, this approach was used to reduce error variance.

The vehicle and test substance formulations were administered once by gavage 2 h prior to ASR assessment. Doses were administered to the adult males via stainless steel gavage needles with ball-tips (22-gauge). In juvenile rats, a flexible small gauge gavage needle was

used. The dose volume for all groups was 5 mL/kg. Individual doses were based on the body weight recorded on the day of dose administration. Technicians scored each animal for clinical observations prior to placing animals into the SR-LAB apparatus for ASR assessment in the juvenile experiment. This was not the case in the adult experiment; however, during the experiment clinical observation data were collected but only after rats were placed in the SR-LAB apparatus. Test intervals were 2, 4, 6, and 8 h post-treatment. Hence, juvenile rats were rated 8 times for clinical observations and adult rats 4 times. PND 15 (16/sex/group) rats were given doses of 0, 1, 2, and 4 mg/kg deltamethrin in 5 mL/kg corn oil, while adults (16 males per group) were given doses of 0, 2, 8, and 25 mg/kg deltamethrin in 5 mL/kg corn oil. Detailed clinical observations and ASR was measured at 2, 4, 6, and 8 hrs for both age groups.

TABLE 3. Study design

Experimental parameter	Dose group (mg/kg bw)			
	Control	Low dose	Mid dose	High dose
Total number of animals/sex/group				
Adult Behavioral testing (Detailed Clinical Observations and Acoustic Startle)	16/Male	16/Male	16/Male	16/Male
Juvenile (PND 15) Behavioral testing (Detailed Clinical Observations and Acoustic Startle)	16/sex[1]	16/sex[1]	16/sex[1]	16/sex[1]

[1] Individual animal data submitted in support of the DER lists data for 17 individuals

3. **Test substance preparation and analysis:** Deltamethrin solutions were made by weighing the needed amount of crystalline deltamethrin and then added to a vial to which reagent-grade corn oil was added to make the concentration for each dose level per each 5 mL/kg. Mixtures were made 24 h prior to the initiation of an experiment. Each solution was placed on an automatic stirrer and continuously mixed overnight.

Results:

Homogeneity analysis: Not reported

Stability analysis: Not reported

Concentration analysis: Not reported

4. **Statistics:** All data were analyzed using SAS programs (SAS Institute, v9.3, Cary, NC). Both descriptive and inferential methods were used to summarize and analyze data. Mixed Linear ANOVA models were used to analyze startle (V_{max}) and body weight using the SAS Proc Mixed program. Where Mixed models involved one between and one within factor, the variance-covariance matrix was tested for best fit using the AICC method. After it was determined that one model fit best, all data were analyzed using the AR(1) (autoregressive-1) covariance matrix. Where significant between x within factor interactions occurred, the SAS Slice-effect ANOVA method in Proc Mixed was used to test for treatment effects at each level of the interaction (i.e., time post-treatment). In both ASR experiments, there were 4 groups; therefore, *a posteriori* pairwise comparisons were used to determine which groups differed from one another. Since the focus was on the effects of deltamethrin, pairwise

comparisons were limited to each treatment group compared to the vehicle control group. Preplanned directional (one-tailed) tests were used for all tests of significance. Statistical significance was set to $p < 0.05$.

E. METHODS/OBSERVATIONS:

1. **Mortality and clinical observations:** Animals were observed before and 2, 2.5, 4, 4.5, 6, 6.5, 8, and 8.5 h after ASR testing for detailed clinical observations. Rats were also checked regularly for mortality and morbidity.

2. **Body weight:** Animals were weighed on the day of ASR testing, prior to dose administration.

3. **Neurobehavioral assessment:**

   a. **Functional Observations Battery (FOB):** Detailed clinical observations for the definitive experiments consisted only of salivation, motility and tremor. Several other detailed clinical observations were evaluated in preliminary studies and were determined to not to have significant results.

   The signs evaluated in pilot experiments were: lacrimation, salivation, ventral wetness, muscle tone, tremors when held, body temperature (subjectively determined, not by thermometer), other handling abnormalities, gait and posture, motility, tremor (visible), clonic seizures, tonic seizures, compulsive licking or biting, writhing, and tail pinch reactivity. Most of these did not generate any data or minimal non-zero ratings. These included perceived body temperature, handling abnormalities, gait, and posture. Several others were seen occasionally but showed so few signs that the data were not reliable. These included lacrimation, seizures (never seen), compulsive licking or biting, and writhing (never seen).

   The detailed clinical observation symptom scale was as follows:
   - Salivation: 0 = none; 1 = slight (i.e., barely perceptible); 2 = moderate (i.e., evident but not severe); 3 = severe (i.e., extensive salivation/wetness around the muzzle, chin, chest or entire ventral surface)
   - Motility: 0 = normal movement/tone; 1 = Slight (i.e., minor movement reduction); 2 = moderate (i.e., clear movement reduction); 3 = severe (i.e., visibly impaired movement)
   - Tremor: 0 = no tremor; 1 = mild/slight (i.e., barely observable); 2 = moderate (i.e., rhythmic muscle movements); 3 = severe/extreme (i.e., whole body tremor)

   b. **Locomotor activity:** Locomotor activity was not evaluated.

   c. **Acoustic Startle:** The preliminary experiments led to the study design for the definitive PND15 and adult acute neurotoxicity experiments. The experiments used ASR and tested at 2, 4, 6, and 8 h post-treatment. ASR sessions were 50 trials mixed with frequency of white noise bursts as the stimulus which lasted for 20 ms with ITIs of 20 s duration following a 5 min acclimation period at the beginning of each session. ASR was assessed in newly purchased and calibrated San Diego Instruments SR-LAB apparatuses (8

identical test chambers with upgraded detection circuit boards for added sensitivity). For adult rats, the calibration setting was 300 units on the sensitivity Vernier but was 700 units for the PND15 pups. The difference in calibration settings provides for similar dynamic range for adults vs. pups but precludes direct comparisons of absolute differences in magnitude of response. The calibration of each chamber was checked prior to each day of testing using an oscillating calibrator. The sound amplitude of the startle stimulus was 120 dB (SPL), and a Quest Sound Level Meter was used to determine the sound level directly under the ceiling speaker where the animal holder was positioned during testing. The sound level meter was calibrated using a Quest standard 90 dB output calibrator.

On the day of an experiment, rats (in their home cage) were placed on transfer racks and moved from their housing room to the Behavioral Suite across the hall and put in a procedure room. Each rat was weighed and gavaged with its designated dose. Once gavaged, rats remained in the room for 1.5 h. The first group of 8 rats was moved to the hall next to the ASR testing room 0.5 h prior to testing. Each rat was removed from its home cage, taken into the ASR test room and scored for the detailed clinical observation set of symptoms, placed in the animal holder and the animal holder door snapped into place and the outer chamber door closed. The process was repeated until all 8 test chambers were filled and the ASR program was started on the computer. The 5 min acclimation period, startle stimulus, and responses on the computer monitor were confirmed by the technician. Once the test session timed out, the technician removed one rat at a time and scored it again for the detailed clinical observation set of symptoms. This process was repeated at 4, 6, and 8 h post-treatment.

The preliminary adult study reported above used 12 rats per group and the effects were not significant possibly because of reductions in ASR across time intervals due to habituation that were in the same direction as the effects of deltamethrin. Therefore, the ASR test sessions were reduced from 100 to 50 trials for the definitive experiment. Additionally, due to the mistake in dosage given, it was not possible to know if the habituation issue or the lower doses caused the non-significant deltamethrin ASR effect in adult rats; therefore, the sample size was increased to 16 rats per group to increase the statistical power. For PND15, 16 litters/dose was used in the definitive experiment. Since litters contain males and females, equal numbers (4 each) of males and females per litter (one pair for each dose group) were tested.

4. **Sacrifice and pathology:** N/A

5. **Positive controls:** N/A

II. DEFINITIVE STUDY RESULTS:

ADULT ANIMALS

A. OBSERVATIONS:

1. **Clinical Signs:** None of the detailed clinical observations rose above moderate in the 2 and 8 mg/kg deltamethrin; however, salivation (1/16) and motility (5/16) peaked at 2.5 h and 4 h

post-treatment, respectively, for 25 mg/kg deltamethrin.

2. **Mortality:** No mortality was observed.

B. **BODY WEIGHT AND BODY WEIGHT GAIN:** No changes in body weight were observed.

C. **ACOUSTIC STARTLE RESPONSE:** Deltamethrin caused decreased ASR at 2, 6, and 8 h post-treatment, yet this dose had a minimal effect on the detailed clinical observations that never rose above mild (i.e., barely detectable) (Figure 10). The decrease was statistically significant at all timepoints for 25 mg/kg deltamethrin and all timepoints except 4 h for 8 mg/kg deltamethrin.

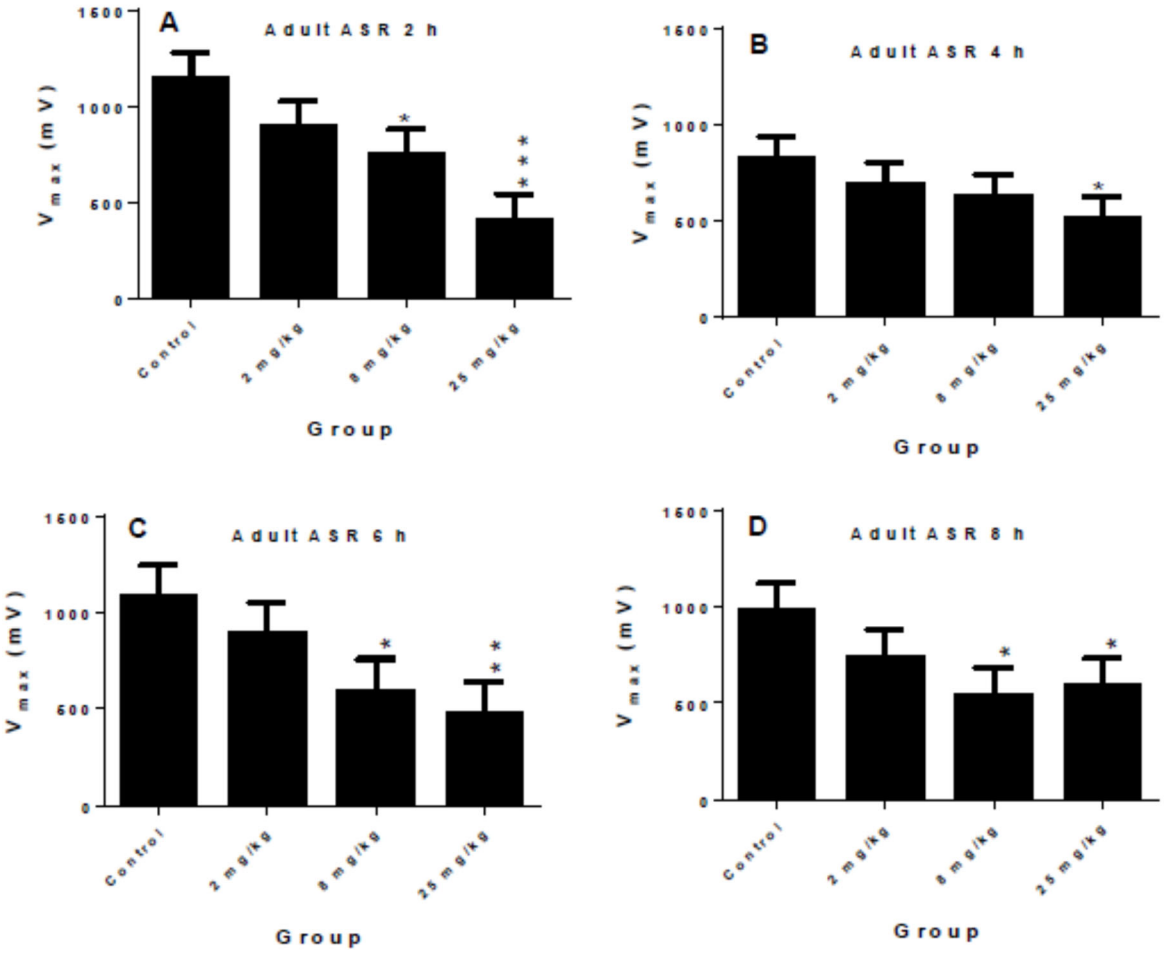


Figure 10. Effects of deltamethrin in adult male SD rats on ASR across time intervals. Data are LS Mean ± SEM. *P<0.05, **P<0.01, ***P<0.001. (MRID 50409301; Figure 20; page 48).

It is concluded that there is no close relationship between the three neurological detailed clinical observation symptoms and deltamethrin effects on ASR. Since there no detailed clinical observation symptoms and not a significant decrease in ASR at the 2 mg/kg deltamethrin dose, 2 mg/kg deltamethrin was identified as the NOAEL for adult deltamethrin exposure.

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JUVENILE (PND15) ANIMALS

A. OBSERVATIONS:

1. **Clinical Signs:** Salivation, motility, and visible tremor were assessed at 2, 4, 6, and 8 h post-treatment. Deltamethrin induced very slight salivation at 6-6.5 h post-treatment after exposure to 2 mg/kg deltamethrin but was most evident in the 4 mg/kg/group. However, salivation was variable and never rose above moderate (Figure 11).

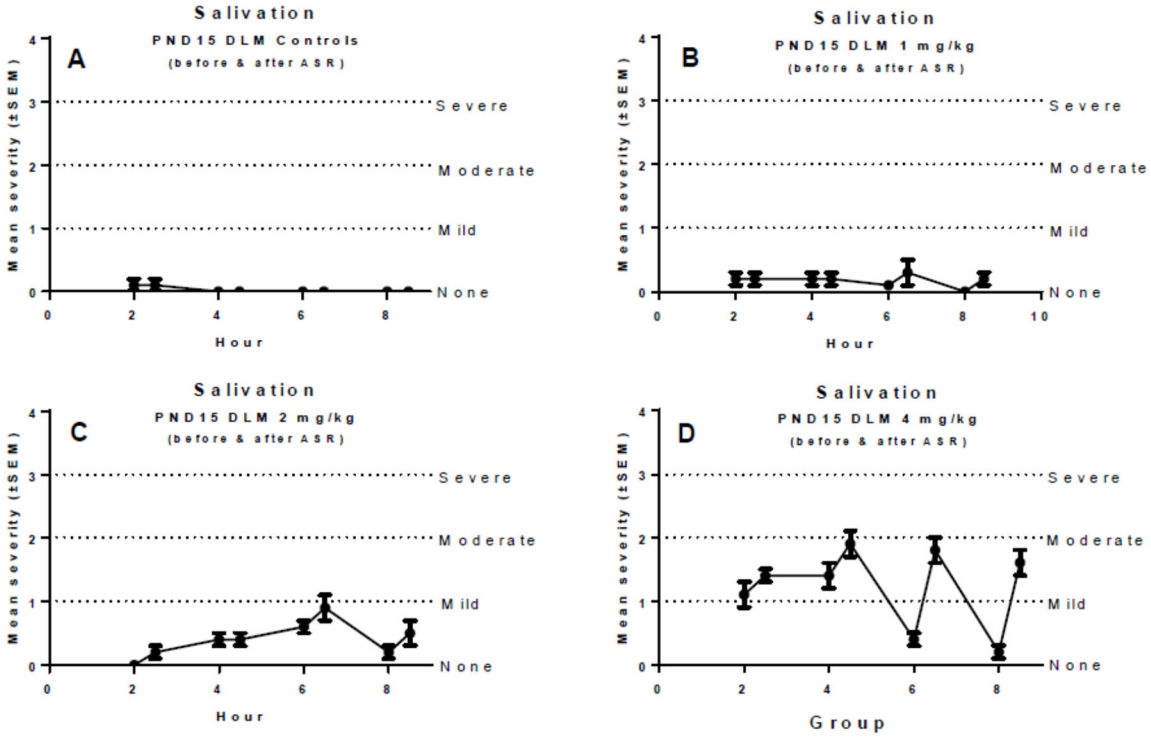

Figure 11. PND15 mean±SEM salivation rating before and after each ASR test session. (MRID 50409301; Figure 21; page 51).

Motility was slightly affected at 1 mg/kg deltamethrin but was more clearly affected at 2 mg/kg deltamethrin (Figure 12). At 4 mg/kg deltamethrin, motility was more affected, rising above moderate at 4.5 h (15/17 for females and male) and remaining above moderate through 8 h post-treatment. Motility approached severe at 4.5 and 6.5 h post-treatment.

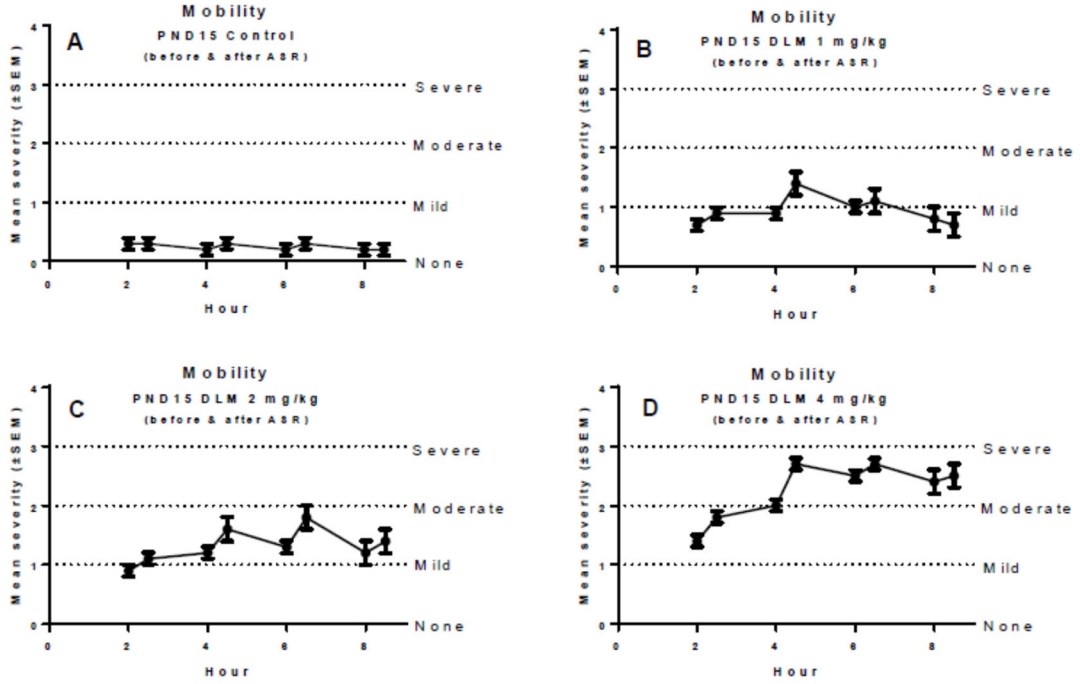

Figure 12. PND15 mean±SEM motility (described as mobility in figure) rating before and after each ASR test session. (MRID 50409301; Figure 22; page 52).

Tremor in PND15 rats was slightly affected at 1 and 2 mg/kg deltamethrin exposure but was evident at 4 mg/kg deltamethrin at 2-4 h post-treatment then rapidly subsided (Figure 13). However, tremor never rose above moderate on average.

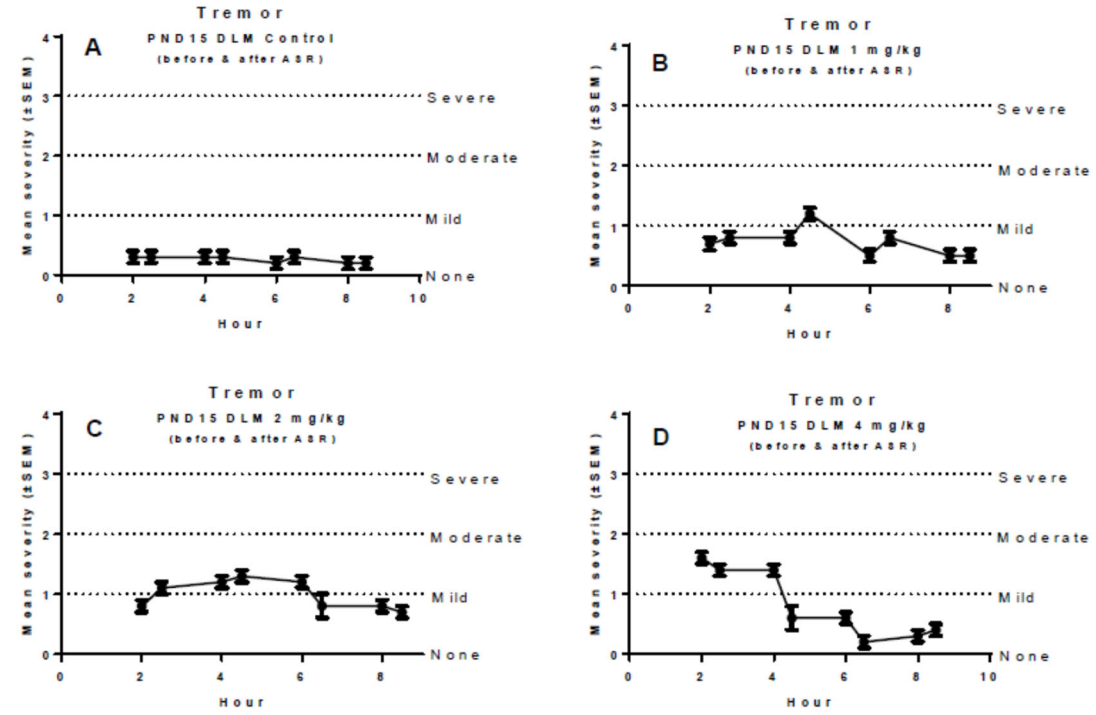

Figure 13. PND15 mean±SEM tremor (visible) rating before and after each ASR test session. (MRID 50409301; Figure 23; page 53).

2. **Mortality:** No mortality was observed.

D. **BODY WEIGHT AND BODY WEIGHT GAIN:** No changes in body weight were observed.

E. **ACOUSTIC STARTLE RESPONSE:** Deltamethrin reduced ASR at all dose levels (1, 2, and 4 mg/kg deltamethrin) at all times (2, 4, 6, and 8 h post-treatment) (Figure 14). Exposure to 4 mg/kg deltamethrin reduced ASR the greatest at 8 h post-treatment suggesting that the effect lasts longer than 9 h. It is not possible to determine if the effect would be needed to determine how long and how large the effects are at longer time intervals. The effect at 1 mg/kg deltamethrin was significant at all time intervals suggesting 1) that this effect extends beyond 8 h post-treatment and 2) that doses below 1 mg/kg deltamethrin are likely to also significantly reduce ASR. It is unlikely that the barely detectable motility and tremor effects noted at 1 mg/kg deltamethrin could account for the ASR effects at this dose given that the motility and tremor were barely perceptible. The results support the view that ASR is more sensitive to deltamethrin than are detailed clinical observations, suggesting that neurological symptoms are not tightly associated with ASR in young rats.

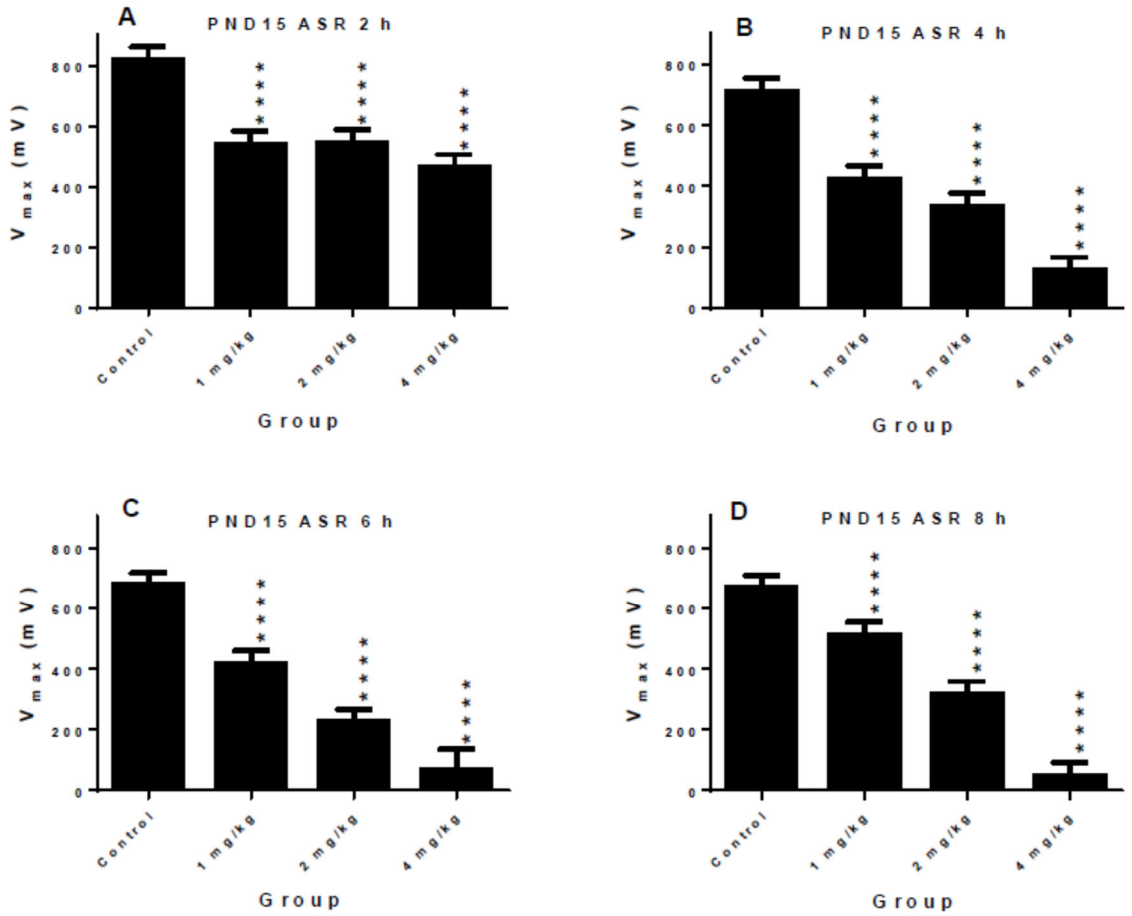

Figure 14. Deltamethrin-induced ASR effects in rats at PND15. ****P<0.0001. (MRID 50409301; Figure 24; page 54).

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The experimental design in these experiments is well suited to demonstrate the direction, magnitude, and time-course of effects of pyrethroids on ASR at multiple dose levels in PND15 and adult rats.

The data firmly establish that PND15 rats are significantly more sensitive to deltamethrin than are adult rats at comparable doses. The experiments also confirmed that PND15 rats are also more sensitive to the neurological (DCO) symptoms induced by deltamethrin than are adults. None of the doses used here in adult or PND15 rats induced the reported CS syndrome. While deltamethrin induced salivation, it did not induce choreoathetosis. Chorea (excessive, spontaneous irregular movements, often of the limbs, including a 'dance-like' gait) and athetosis (wriggling, squirming, or writhing movements) were not seen in any animals in the adult or PND15 rats. Rather a loss of coordination was observed at higher doses and loss of balance because of the loss of limb control. Hence, the "C" of the reported CS syndrome was never observed.

B. REVIEWER COMMENTS: The EPA reviewer concurs that treatment with deltamethrin decreased the acoustic startle response in PND 15 and adult rats. However, appropriate doses for PND 15 pups were not established, for there were adverse effects at all doses tested which in turn did not allow study authors to establish a clear time to peak effect, or post-treatment interval for testing. The dose selection for the definitive study was not reviewed by the Agency prior to the initiation of the study; the Agency would have preferred to have participated in the dose selection process. In the preliminary study, significantly decreased ASR was observed at 2 h post-treatment in the 1.8, 2.4, and 3 mg/kg deltamethrin groups. Additionally, at 8 h post-treatment, the 0.6 mg/kg deltamethrin groups showed a trend, so the reviewer does not understand why lower doses were not used for the definitive study when an effect was observed in the preliminary study at doses lower than those chosen for the definitive study. The reviewer does agree that PND 15 pups were clearly more sensitive to the neurotoxic effects of deltamethrin based on clinical observations.

The Agency reanalyzed the both the juvenile and adult datasets (D448563, 9/25/2018). The juvenile data was reanalyzed by selecting the variance-covariance matrix that produced the lowest AIC value among different variance-covariance matrices (AR(1), CS, and UN). The data analysis explained in the DER instead used the variance-covariance matrix = AR(1) and assumed to be the same for different dose groups. Further, the Agency's analysis also appropriately addressed the unequal variances among dose groups (variance heterogeneity). Dunnett's test was used to correct the p-values from multiple comparisons. The reanalysis did not alter the initial conclusions, and all the dose groups in both male and female groups were significantly different from the control group. In the adult data, regression diagnostics indicated that the assumption of normality in the residuals was mildly violated. A square-root transformation would normalize the data. However, as shown in the sensitivity data analysis, a square-root transformation of V_{max} prior to the data analysis would not alter or affect the conclusions of statistical significance of the treatment effects; therefore, the data was not transformed during reanalysis. After reanalysis, only the high dose group of adult rats (25 mg/kg) was significantly different from the control and only at 2 hours and 4 hours following the treatment.